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Lipopolysaccharide structure impacts the entry kinetics of bacterial outer membrane vesicles into host cells

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Supporting Information for

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Supporting Materials and Methods

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SUPPORTING MATERIALS AND METHODS

Nanoparticle tracking analysis

After purification, OMV samples were diluted $1x10^{-6}$ in filtered sterile PBS. Particle diameter and concentration were measured using the Nanosight LM10 particle tracking analysis, with a minimum of 100 tracks per sample, performed in triplicate. Camera shutter 1495 and gain of 450 were used, and size distribution scatter plots were created using GraphPad Prism. Size distribution was analysed using analysis of variance (ANOVA) with a Brown Forsythe test for equal variance.

Measurement of ζ-potential as an indicator of OMV surface charge

700 µl of OMV preparations were analysed using a Zeta Sizer (Malvern Instruments) and data from an average of 30 readings/sample were acquired at 37 °C and means were plotted.

Visualization of outer membrane vesicles by Transmission Electron Microscopy

10µl of isolated outer membrane vesicles in sterile deionized distilled water were added to 400-mesh copper grids, and negatively stained with 4% uranyl acetate for 2 min. Samples were then observed using a Jeol 1200Ex transmission electron microscope (Birmingham Electron Microscopy Facility) with an acceleration of 75kV.

SUPPORTING FIGURES

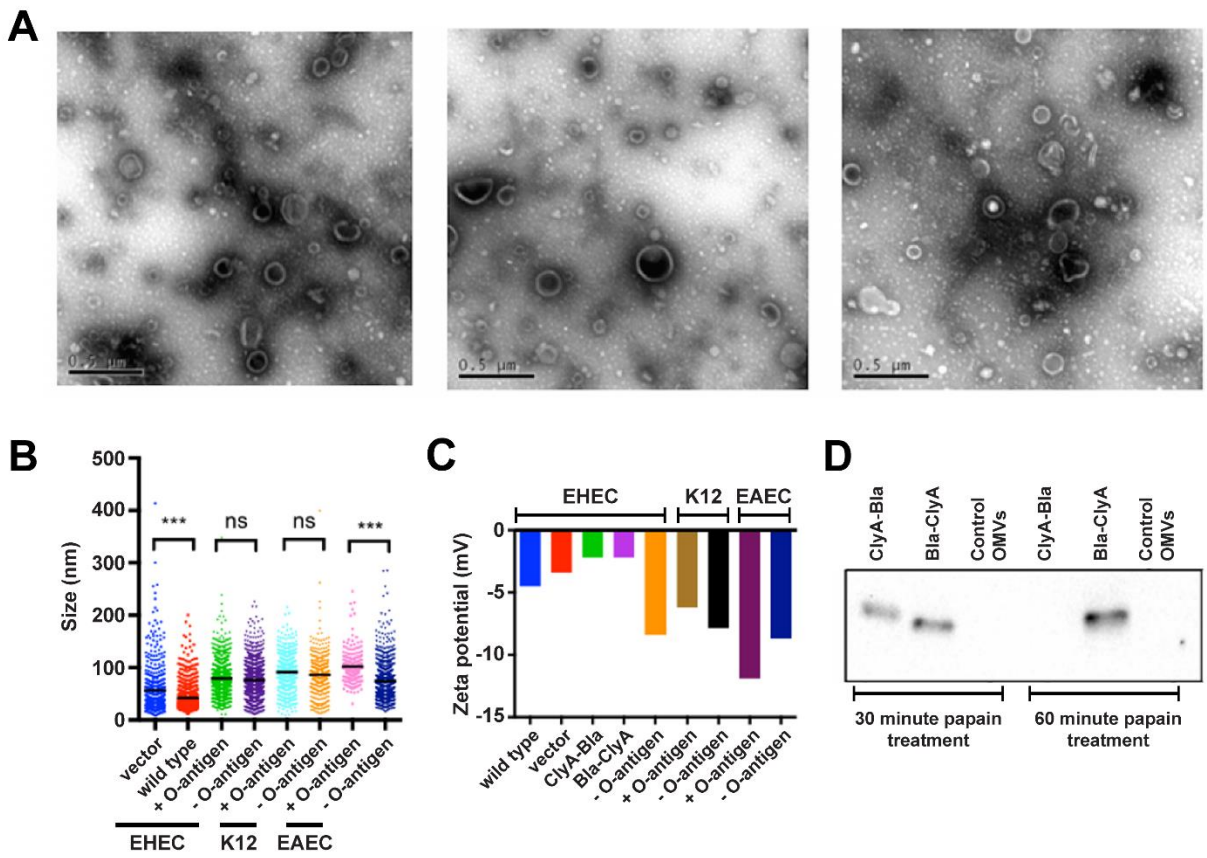

Figure S1. Morphology, size, charge and probe orientation of reporter OMVs. (A) Electron micrographs of negative stained OMV fractions from EHEC wt (left image) or EHEC ClyA-Bla (centre and right images). Scale bars, 0.5 µm. (B) Isolated OMVs were diluted $1x10^{-6}$ fold and nanoparticle tracking analysis was used to determine the size distribution. Black lines represents median size from at least 200 tracks acquired per sample. Statistical significance was determined by ANOVA, with a Brown Forsythe test to determine equal variance. (***) $p \leq 0.005$, (ns) not significant. (C) ζ-potentials of isolated OMVs. Values represent means from 30 readings per sample. Only means are displayed since individual readings are not accessible instrumentally. (D) OMV fractions from EHEC expressing Cly-Bla, Bla-ClyA or carrying empty vector were treated with papain for 30 or 60 minutes, and used for Western Blotting with α-Bla antibody.

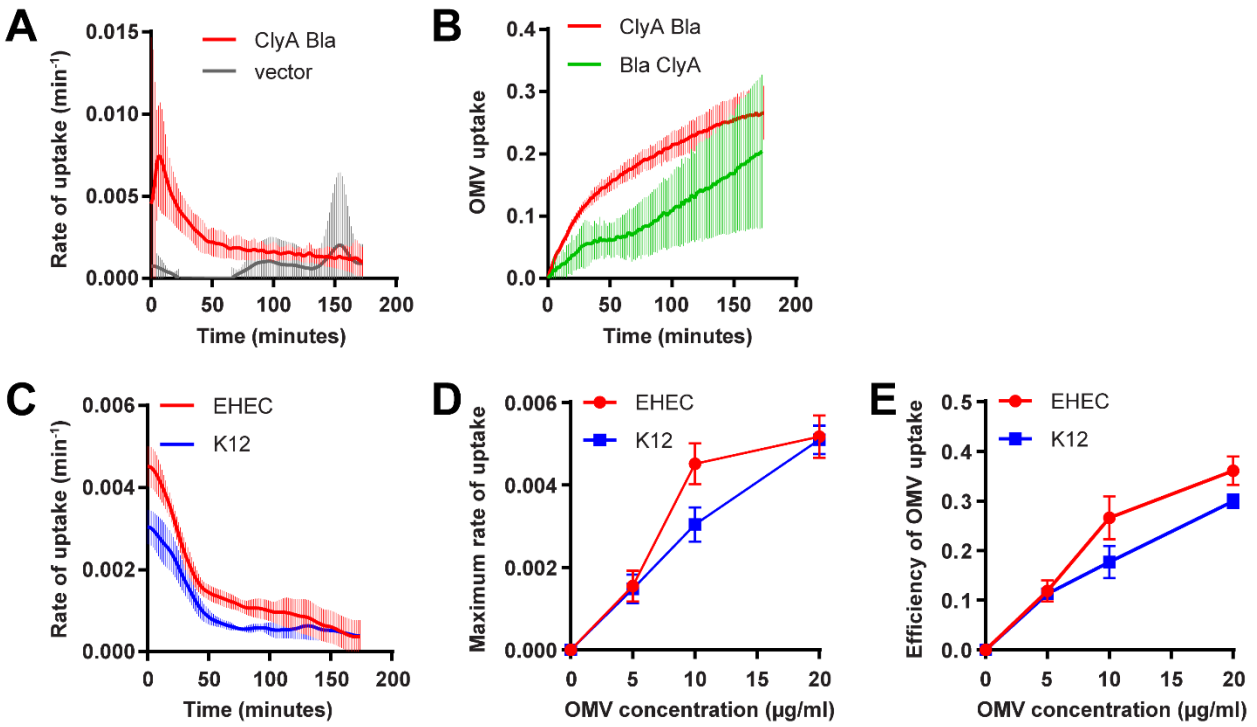

Figure S2. Rates of uptake/dismantling and concentration dependency of uptake kinetics for OMVs. (A) CCF2-AM loaded Hela cells exposed to EHEC OMVs carrying ClyA-Bla (red), or empty vector (grey) at an MOI of 1000 for 3 h. Rate of uptake over time was extracted from data in Figure 2A and data shown are means ± stdev (n=3). (B) FRET change upon exposure of Hela cells to EHEC OMVs carrying ClyA-Bla (reporting on exposure to OMV surface to cytoplasm) or Bla-ClyA (reporting on exposure of luminal cargo to cytoplasm). (C) Hela cells were exposed to EHEC or K12 ClyA-Bla OMVs at an MOI of 1000 for 3 hours. Rates of uptake over time were extracted from data in Figure 3A and are means ± stdev (n=3). (D) Experiments were repeated as above but using different OMV concentrations (0-20 µg/ml of protein, corresponding to an MOI of 0- 2000), and maximum rates (D) and efficiency of uptake (E) determined as described above. Data are means ± stdev (n=3).

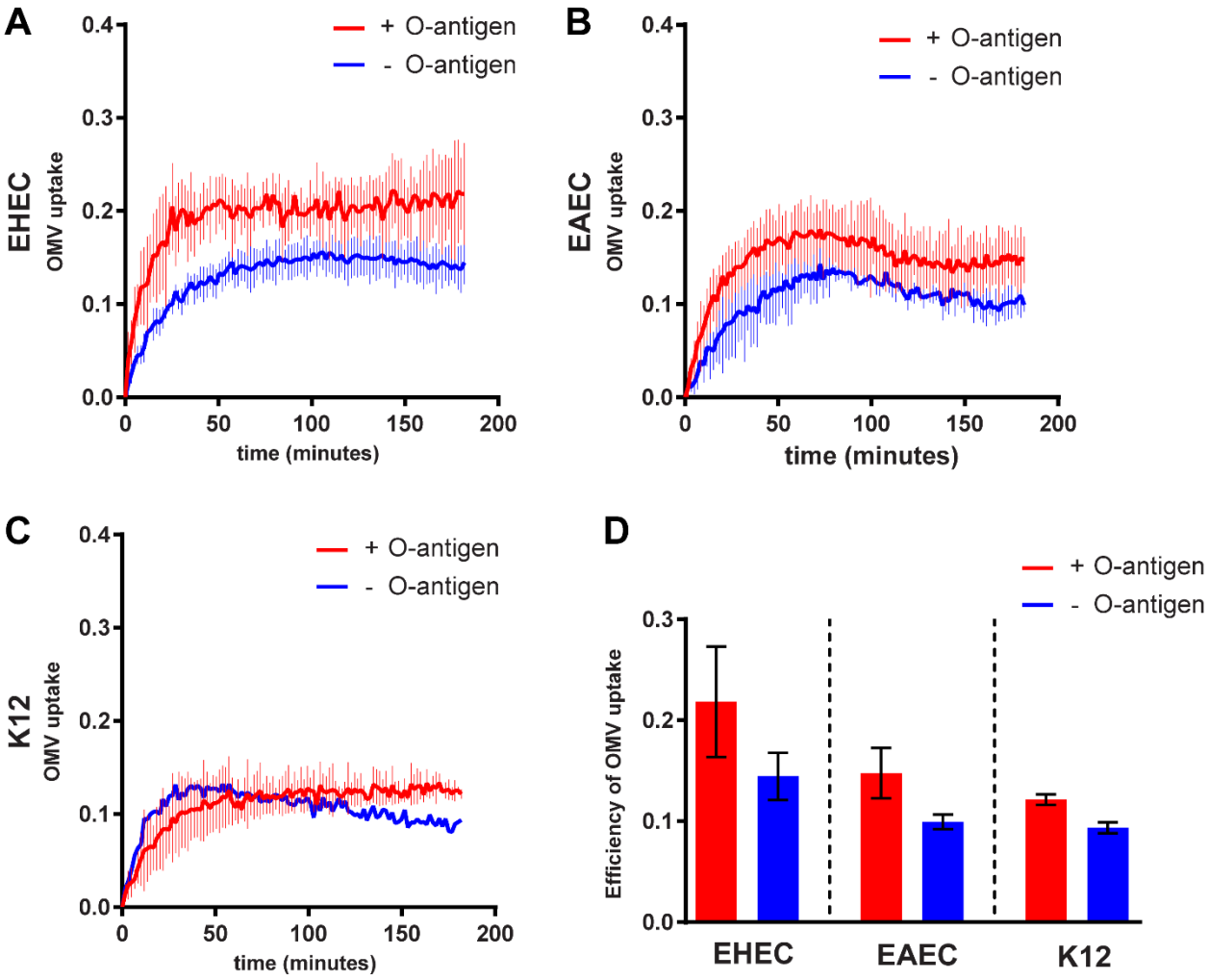

Figure S3. Uptake for OMVs from serotypes O157, O42 and O16 with or without O antigen. CCF2-AM loaded RKO intestinal epithelial cells were exposed to OMVs from EHEC O157 (A), EAEC O42 (B), and K12 O16 (C), with O antigen (red) and without O antigen (blue), at an MOI of 1000 for 3 hours. FRET changes (blue/green fluorescence, A-C) and efficiency of uptake (total change over three hours, D) are shown as means ± stdev (n=3).

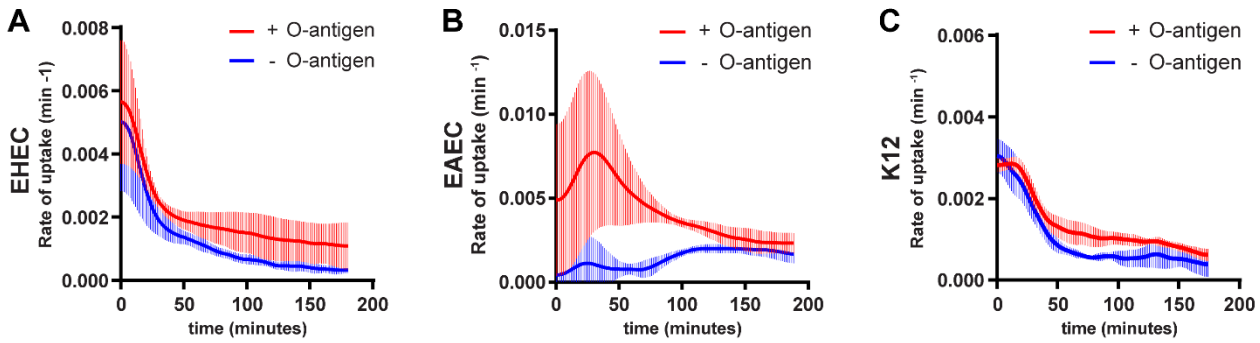

Figure S4. Rates of uptake for OMVs from serotypes O157, O42 and O16 with or without O antigen. CCF2-AM loaded Hela cells were exposed to OMVs from EHEC O157 (A), EAEC O42 (B), and K12 O16 (C), with O antigen (red) and without O antigen (blue), at an MOI of 1000 for 3 hours. Polynomials were fitted to each data set using the cubic spline function csaps in Matlab. Numerical estimates of the gradients of the resulting polynomials were determined using the gradient function. Data shown are means ± stdev (n=3).

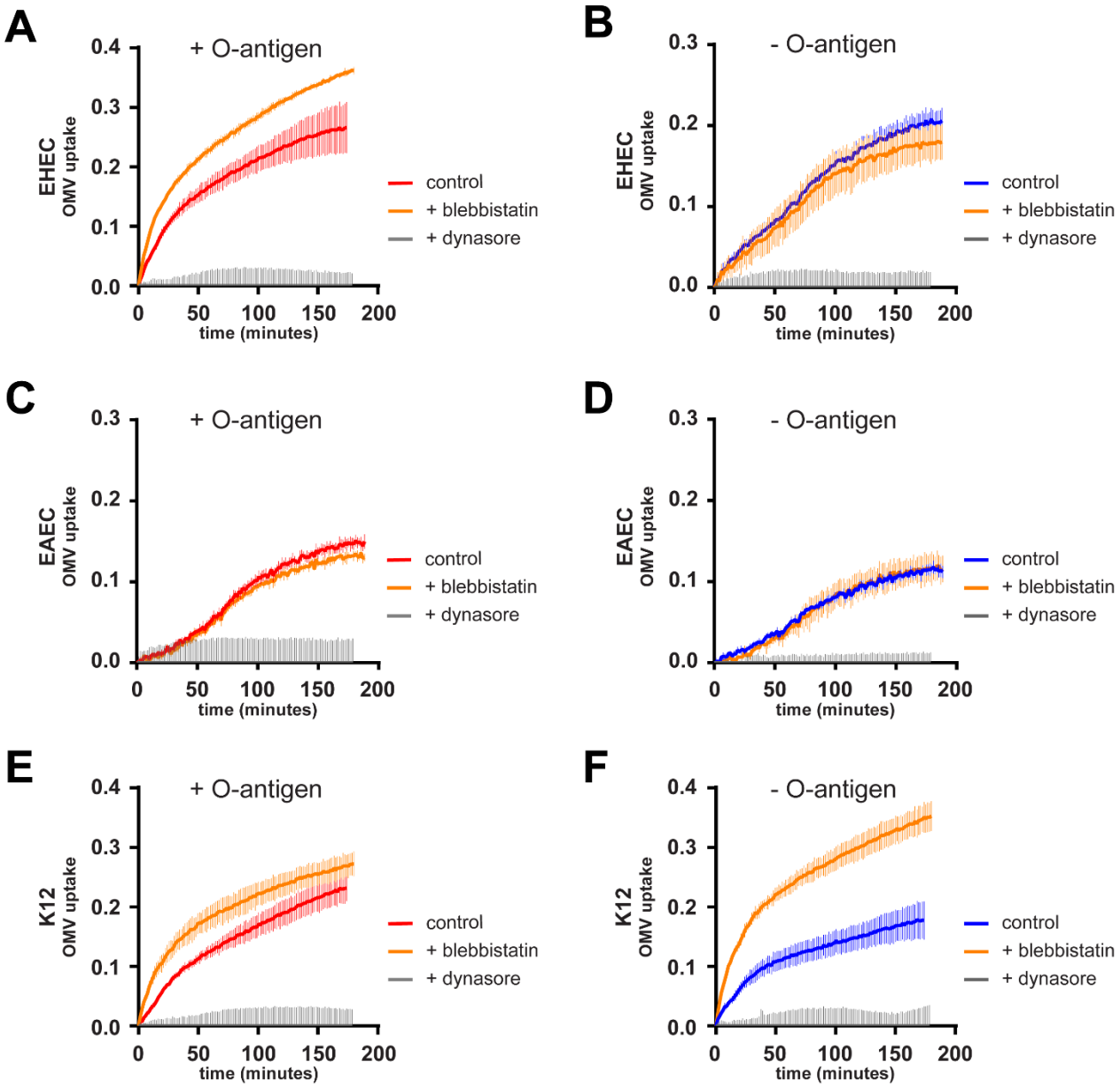

Figure S5. Effect of blebbistatin and dynasore on uptake of OMVs. Hela cells were either left untreated or pre-treated 80 uM Dynasore for dynamin inhibition (grey), or 20 uM blebbistatin for macropinocytosis inhibition (orange) for 1h at 37 °C and exposed to ClyA-Bla OMVs isolated from EHEC (A, B), EAEC (C, D), or K12 (E, F) at an MOI of 1000 for 3 hours. The FRET signal (ratio of blue:green fluorescence) over time was plotted as mean ± stdev (n=3).

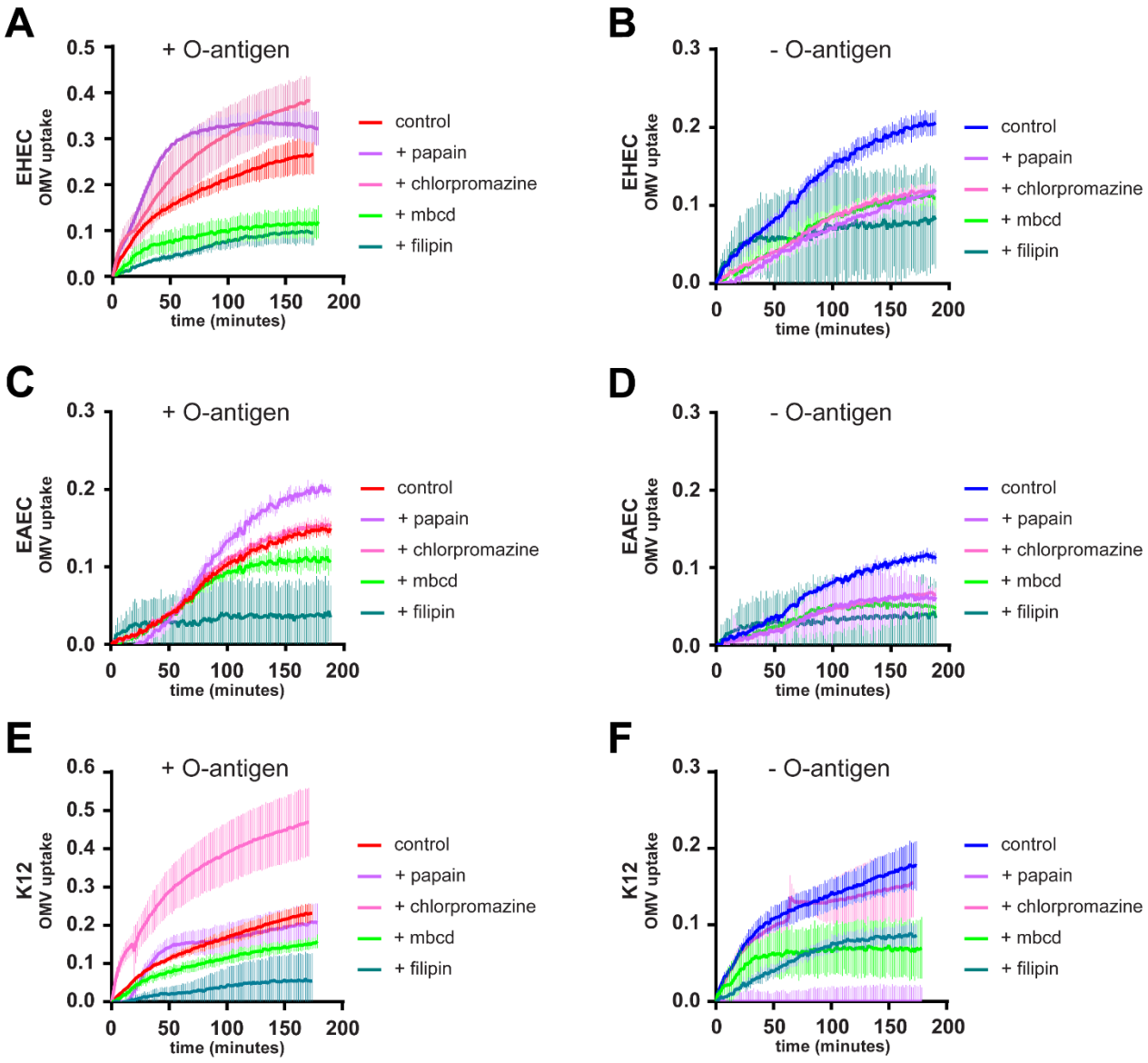

Figure S6. Effect of pharmacological treatments on OMV uptake. Hela cells were either left untreated or pre-treated with 5 ug/ml papain (lilac), 1 ug/ml chlorpromazine (pink), 5mM methyl-β-cyclodextrin (light green) or 1µg/ml filipin (turquoise) and exposed to ClyA-Bla OMVs isolated from EHEC (A, B), EAEC (C, D), or K12 (E, F) at an MOI of 1000 for 3 hours. The FRET signal (ratio of blue:green fluorescence) over time was plotted as means ± stdev (n=3).